

Claims:

1. A method for the non-invasive early detection of colon cancer and/or intestinal cancer precursor cells by means of mutational analysis of the genes for APC, K-ras, β -catenin and B-raf in a sample, characterized in that

the method comprises the following steps:

- collecting a stool and/or tissue sample,
- homogenizing the sample,
- obtaining DNA from the sample,
- performing an amplification reaction in the genes for APC, K-ras, β -catenin and B-raf, using the primers

s1 TTGCAGTTATGGTCAATACCC
as1 GTGCTCTCAGTATAAACAGGATAAG
s2 CCTCAAAAGGCTGCCACTTG
as2 CTGTGACACTGCTGGAACCTCGC
s3 AGCACCCCTAGAACCAAATCCAGCAG
as3 TGGCATGGTTGTCCAGGGC
s4 ACAAACCATGCCACCAAGCAGA
as4 GAGCACTCAGGCTGGATGAACAAG
s5 TTCCAGATGCTGATACTTTA
as5 CTGAATCATCTAATAGGTCC

for APC, the primers

s CTGGTGGAGTATTGATAGTG
as TCTATTGTTGGATCATATTC

for K-ras, the primers

s CTGATTGATGGAGTTGGAC
as CTTGAGTGAAAGGACTGAGA

for β -catenin, and the primers

s TGTATCACCCTCTCCATATC
as GCATTCTGATGACTTCTGGT

for B-raf,

wherein amplification products are formed, and

- performing a mutational analysis in the amplification products.
- 2. The method according to claim 1,
characterized in that
the detection of mutations in selected sections of the genes for APC, K-ras, β -catenin and B-raf is effected by means of a DNA chip, said DNA chip including probes for APC, K-ras, β -catenin and B-raf from those regions of the above-mentioned genes that are flanked by the primer sequences specified in claim 1.
- 3. The method according to claim 1 or 2,
characterized in that
the APC, K-ras, β -catenin and B-raf genes are accumulated from total DNA by hybridizing sequence-specific biotinylated oligonucleotides with the genes for APC, K-ras, β -catenin and B-raf using coupling of the biotin residue to streptavidin and subsequent separation via magnetic particles.
- 4. The method according to claims 1 to 3,
characterized in that
amplification products, especially PCR products, are separated in an agarose gel for control purposes prior to purification.
- 5. The method according to any of claims 1 to 4,
characterized in that
the mutational analysis of the PCR products is effected using electrophoretic techniques, preferably SSCP, alternatively by means of a chromatographic procedure, preferably an HPLC-based procedure.

6. The method according to the preceding claim,
characterized in that
detected mutagenic conformations of a single strand are
isolated and optionally sequenced.

7. Primer sequences selected from the group comprising:
the primers

s1 TTGCAGTTATGGTCAATAACCC
as1 GTGCTCTCAGTATAAACAGGATAAG
s2 CCTCAAAAGGCTGCCACTTG
as2 CTGTGACACTGCTGGAACCTTCGC
s3 AGCACCCCTAGAACCAAATCCAGCAG
as3 TGGCATGGTTGTCCAGGGC
s4 ACAAACCATGCCACCAAGCAGA
as4 GAGCACTCAGGCTGGATGAACAAG
s5 TTCCAGATGCTGATACTTTA
as5 CTGAATCATCTAATAGGTCC
or alternatively
s2 GAATCAGCTCCATCCAAGT
as2 TTTCTGCTATTGCAGGGT
for APC, the primers
s CTGGTGGAGTATTGATAGTG
as TCTATTGGATCATATTG
for K-ras, the primers
s CTGATTGATGGAGTTGGAC
as CTTGAGTGAAGGACTGAGAA
for β -catenin, and the primers
s TGTATCACCATCTCCATATC
as GCATTCTGATGACTTCTGGT
for B-raf.

8. Use of the primer sequences according to claim 7 in mu-
tational analysis, especially in the analysis of the
APC, K-ras, β -catenin and B-raf genes.

9. A kit, comprising primers selected from the group comprising:

the primers

s1 TTGCAGTTATGGTCAATAACCC
as1 GTGCTCTCAGTATAAACAGGATAAG
s2 CCTAAAAGGCTGCCACTTG
as2 CTGTGACACTGCTGGAACCTTCGC
s3 AGCACCCCTAGAACCAAATCCAGCAG
as3 TGGCATGGTTGTCCAGGGC
s4 ACAAAACCATGCCACCAAGCAGA
as4 GAGCACTCAGGCTGGATGAACAAG
s5 TTCCAGATGCTGATACTTTA
as5 CTGAATCATCTAATAGGTCC

or alternatively

s2 GAATCAGCTCCATCCAAGT
as2 TTTCTGCTATTGCAGGGT

for APC, the primers

s CTGGTGGAGTATTGATAGTG
as TCTATTGTTGGATCATATTG

for K-ras, the primers

s CTGATTGATGGAGTTGGAC
as CTTGAGTGAAGGACTGAGAA

for β -catenin, and the primers

s TGTATCACCATCTCCATATC
as GCATTCTGATGACTTCTGGT

for B-raf,

and optionally information relating to combining the contents of the kit.

10. Use of the kit according to claim 9 in the detection of colon cancer and/or colon cancer precursor cells.